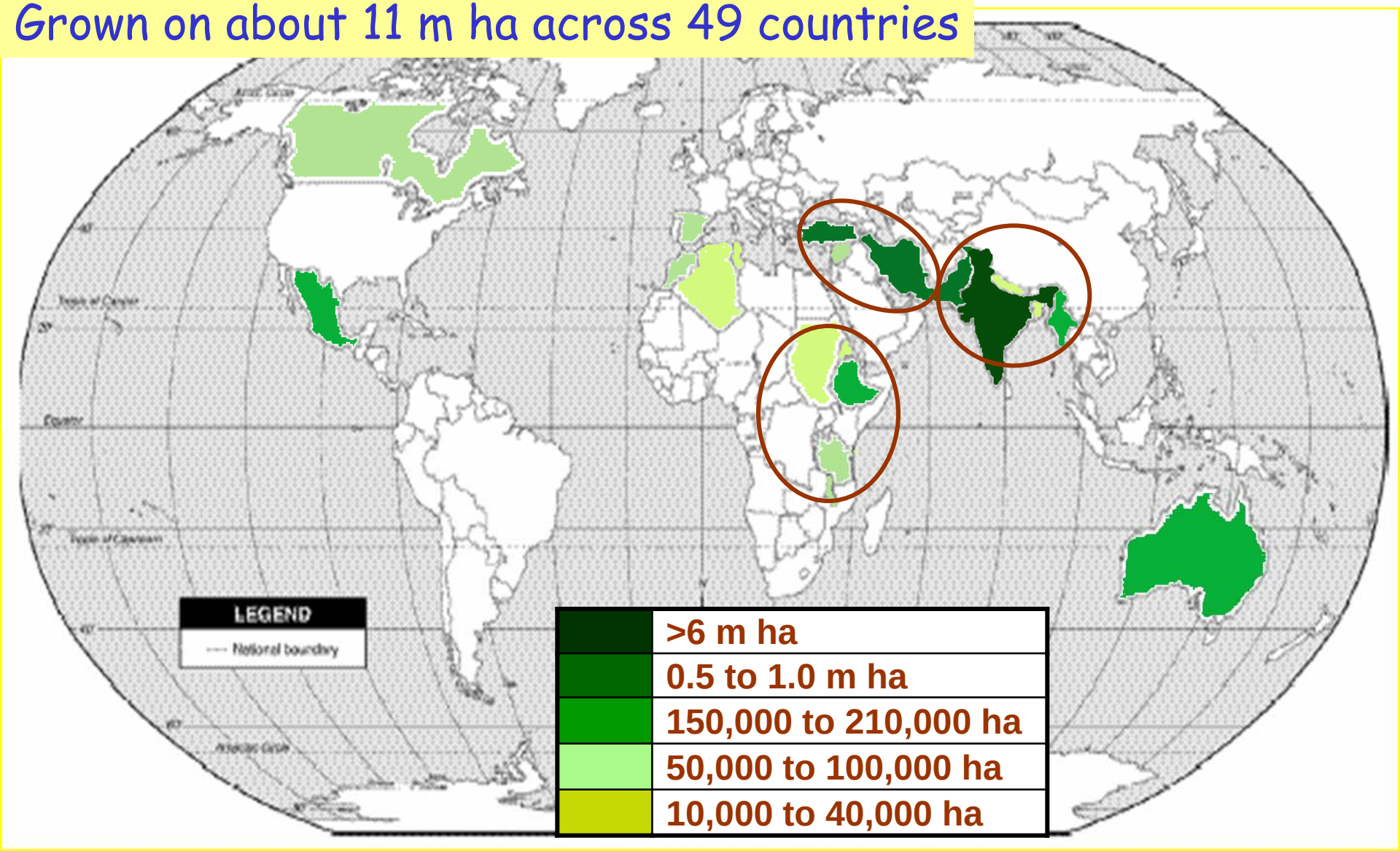
DEVELOPING GENETIC AND GENOMIC RESOURCES IN CHICKPEA FOR IMPROVING CROP PRODUCTIVITY IN SUB-SAHARAN AFRICA AND ASIA

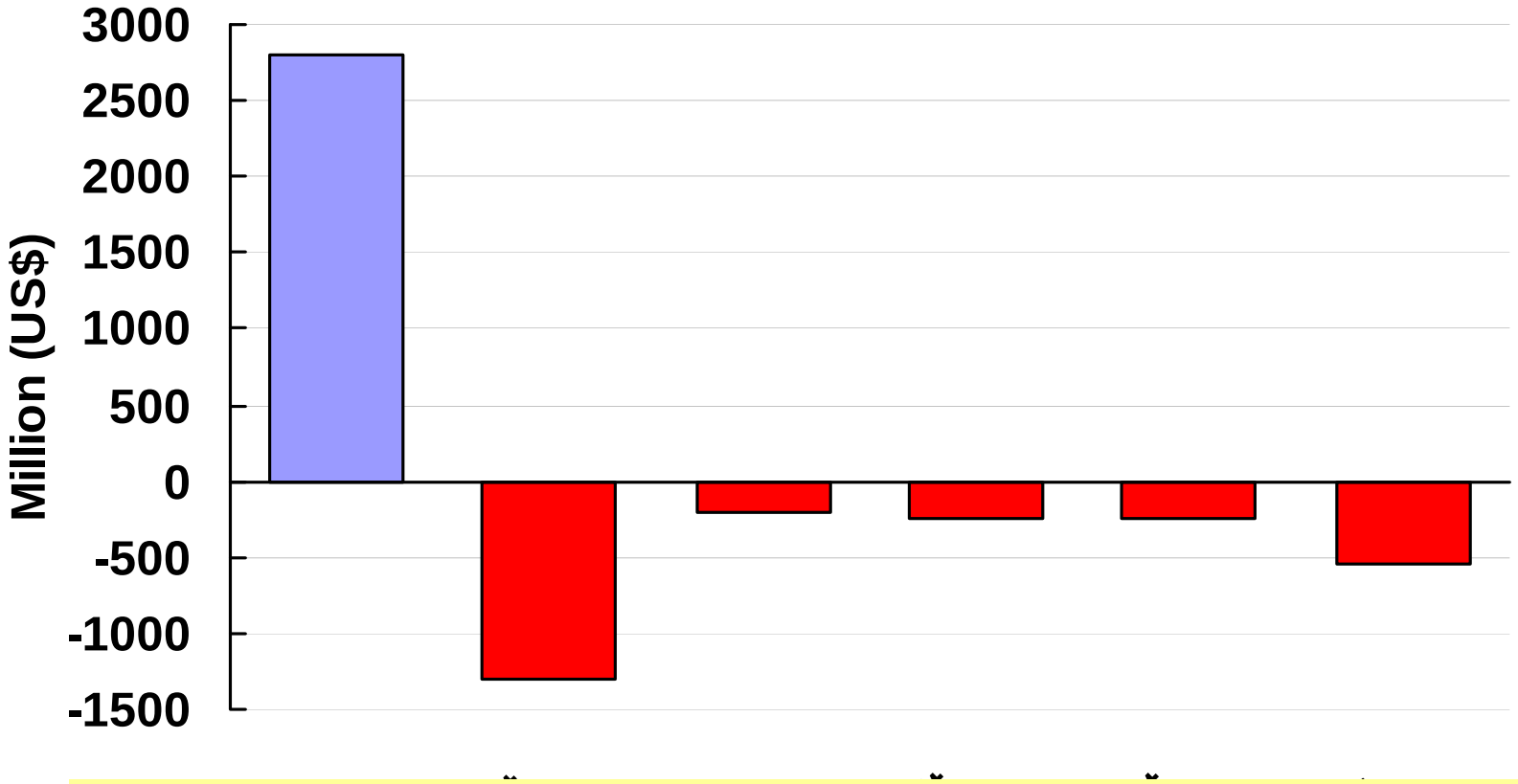
Global distribution of chickpea area

Grown on about 11 m ha across 49 countries



Grown in marginal environments in Sub-Saharan Africa & Asia

Production constraints



Need for improving chickpea crop for drought tolerance and insect resistance

Root traits for drought tolerance

RLD (cm cm^{-3})	Moderate drought				Severe drought (2000/01)			
	YLD		HI		YLD		HI	
0-15cm	0.344		0.354		0.442		0.404	
15-30cm	0.699	*	0.672	*	0.718	**	0.709	**
30-45cm	0.406		0.544		0.779	**	0.616	*
45-60cm	0.405		0.496		0.576	*	0.355	
Total	0.613	*	0.681	*	0.659	*	0.565	

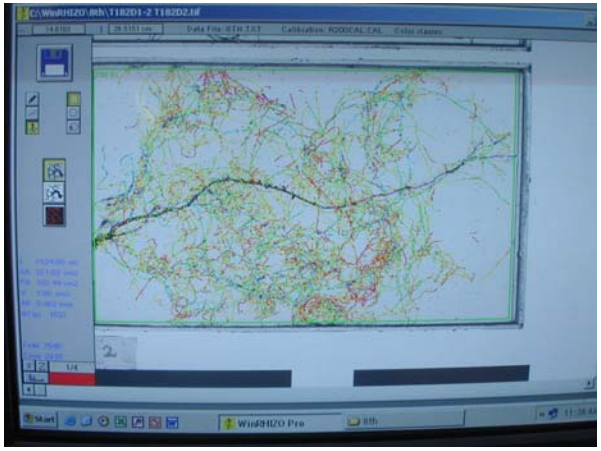
RLD = Root Length Density, YLD= Yield, HI= Harvest Index

Kashiwagi et al. 2006, Field Crops Research 95: 171-181

Large scale root trait phenotyping (PVC cylinder system)

Field capacity soil vs Field conditions: 0.023 ns

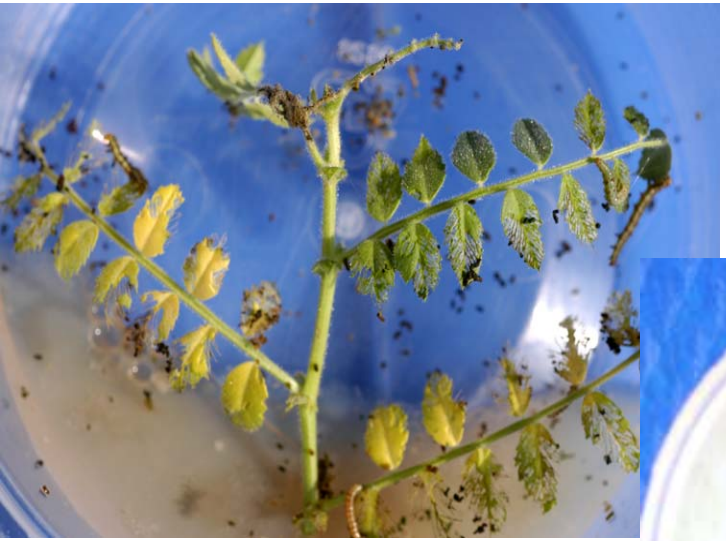
70% field capacity soil vs Field conditions: 0.634*



Screening for *Helicoverpa* resistance (under natural infestation in field)

Data recorded on
- *damage rating*
- *larvae counted on plants*
- *overall resistance score*
- *pod damage*
- *grain yield*

Screening for *Helicoverpa* resistance (detached leaf assay)



Susceptible

Resistant

- cut branches placed o*n agar plate*
- *counted number of 10 larvae released on cut branches*
- *observations recorded on leaf damage, larval survival and weight gained by insects*

5 Activities

Activity 1: Develop germplasm for genetic studies and modern breeding (GERMPLASM)

Activity 2: Generate genomic resources for genetic studies and modern breeding (GENOMIC RESOURCES)

Activity 3: Identify molecular markers and genes for biotic stress resistance (INSECT RESISTANCE)

Activity 4: Identify molecular markers and genes for drought tolerance (DROUGHT TOLERANCE)

Activity 5: Improve locally adapted germplasm for target traits through modern breeding (MODEREN BREEDING)

Strategies being followed

Developing genomic resources

- critical mass of molecular markers: SSRs/DArTs/SNPs

Phenotyping of germplasm collections

- reference set (300) for root traits, harvest index and insect resistance

Mapping of drought and insect resistance

- interspecific mapping population for insect resistance
- intraspecific mapping populations for root traits
- genetic mapping and QTL analysis

Modern breeding

- MABC and MARS

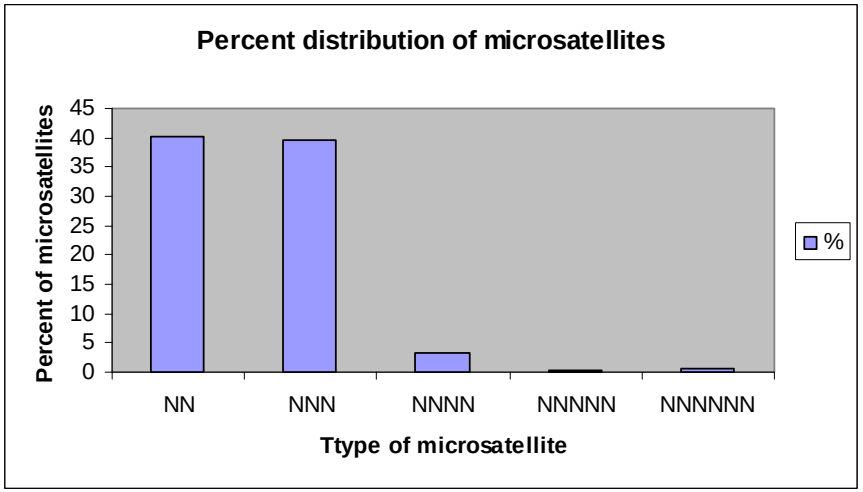
Genomic resources…

Genomic resources: gSSRs

Microsatellite (GA and TAA) enriched genomic library constructed from ICC 4958 genotype in pGEM-3Zf (+) vector

In co-operation with:
Uni Frankfurt/GenEx pro, Germany

SSR +ve clones generated	**288**
Sequences generated	**438**
Sequence data examined	**273 kbp**
Total SSRs identified	**615**
SSR containing sequences	**286**
Primers designed for non-redundant SSRs	**~311**



Primer aliquots already distributed:

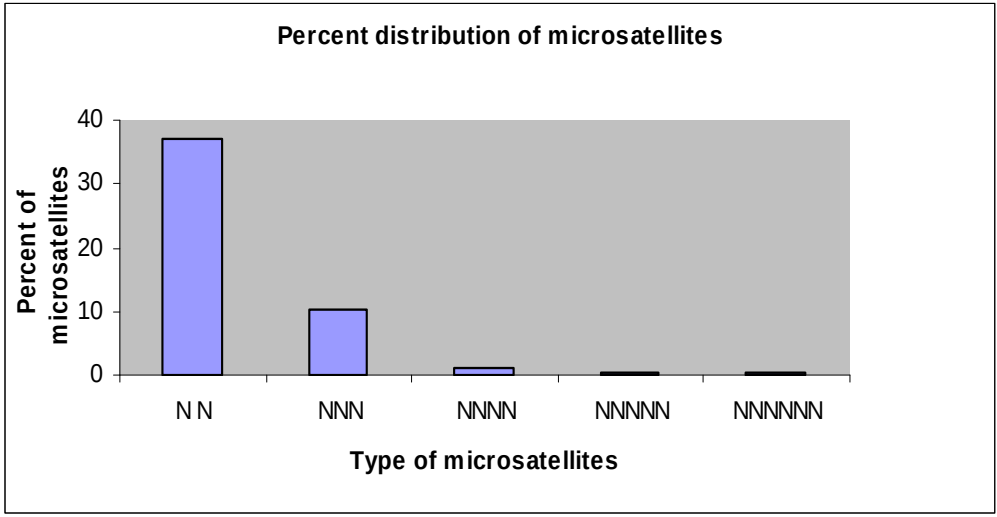
Washington State University, Pullman, USA (Fred Muehlbauer)
ACNFP, Murdoch University, Perth, Australia (Richard Oliver)
National Chemical Laboratory, Pune, India (Vidya Gupta)
National Research Centre on Plant Biotechnology, New Delhi, India (R Srinivasan)
Sardar Vallabh Bhai Patel University, Modipuram, Meerut (Rajendra Kumar)
Indian Agricultural Research Institute (IARI), New Delhi (Jitendra Kumar)

Genomic resources: BES-SSRs

BAC library (CAA1Ba), constructed from ICC 4958, was sequenced and BAC-ends were used for mining SSRs and marker development

In collaboration with:
University of California, Davis, CA, USA
(Doug Cook, NSF project)

Sequences examined	46270
Total size of examined sequences (kb)	33,217
Identified SSRs	6845
Sequences containing SSRs	5123
Frequency of SSRs	1/4.85 kb
Primers designed for non redundant SSRs	1344



Novel SSRs in chickpea

	SSR enriched library	BAC-end sequences	
Number of clones	288	25,000	
Sequences surveyed	457	46,270	
Amount seq Data (bp)	286,718	33,217,120	
SSRs identified	643	6,845	
SSR frequency	1 / 445 bp	1/ 4.9 kb	
Primers designed	311	4,964	
Primers synthesized	311	1,344	
Primers tested	311	1,344	**total 1,655**
Primers amplified	225	1,214	**total 1,439**

Total SSR markers in chickpea

Number of SSRs	References
28	Huettel et al. 1999
174	Winter et al. 1999
233	Lichtenzveig et al. 2005
280	Choudhary et al. 2006, Sethy et al. 2003, 2006a, b, Bhatia unpublished
311	ICCM series (SSR-enriched lib.)
1344	CaM series (BAC-end seqs)

Total- 2370 SSR markers
(70% developed under this project)

Genomic resources: DArTs/ SNPs

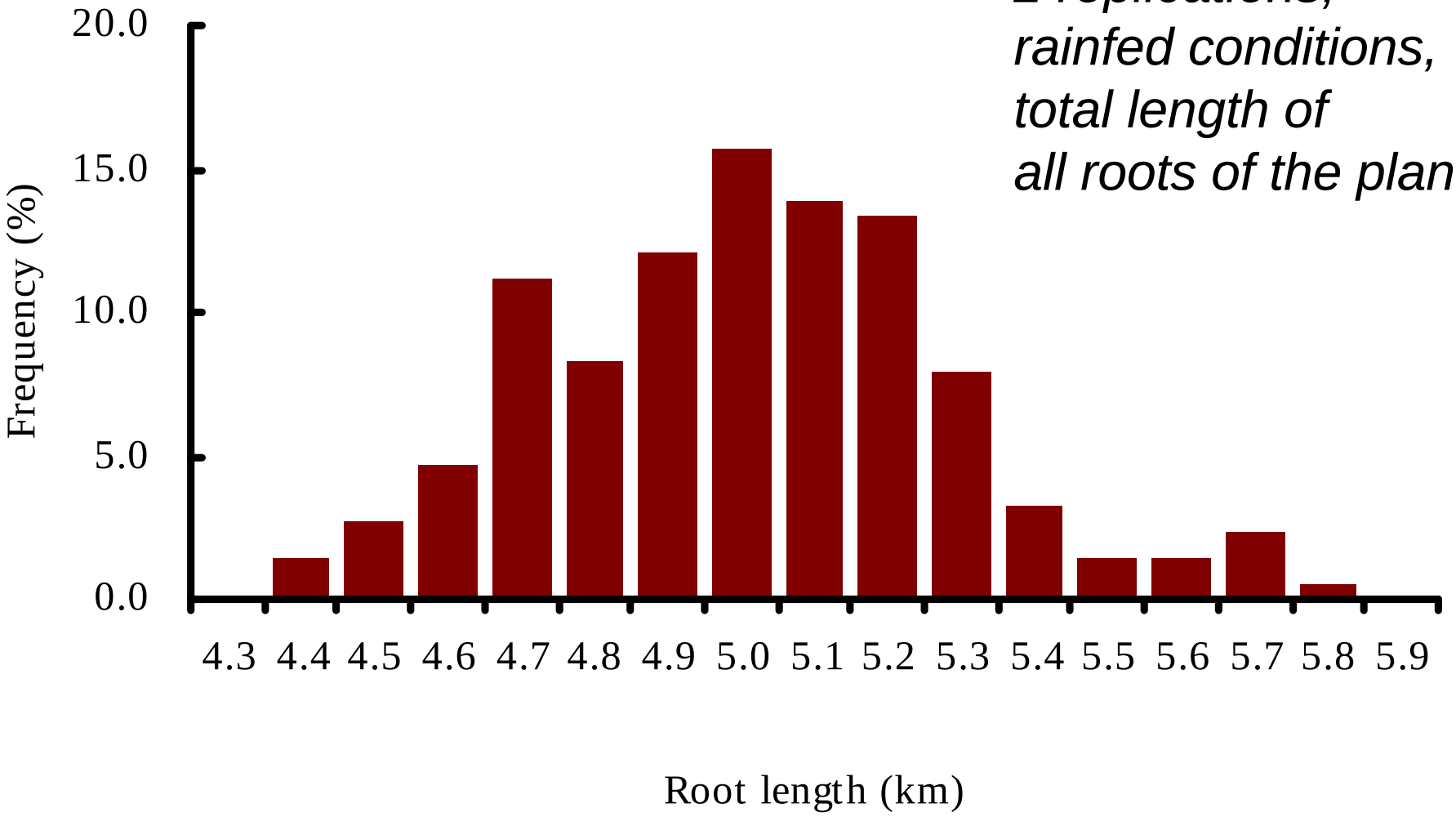
- An expanded DArT array of about 18,000 genomic DNA clones being developed that includes
  - 96 genotypes
  - parental genotypes of mapping populations,
  - diverse accessions from reference collection
  - wild *Cicer* species used for introgressions

- SNP Illumina array being developed in collaboration with Objective 5 (Doug Cook)

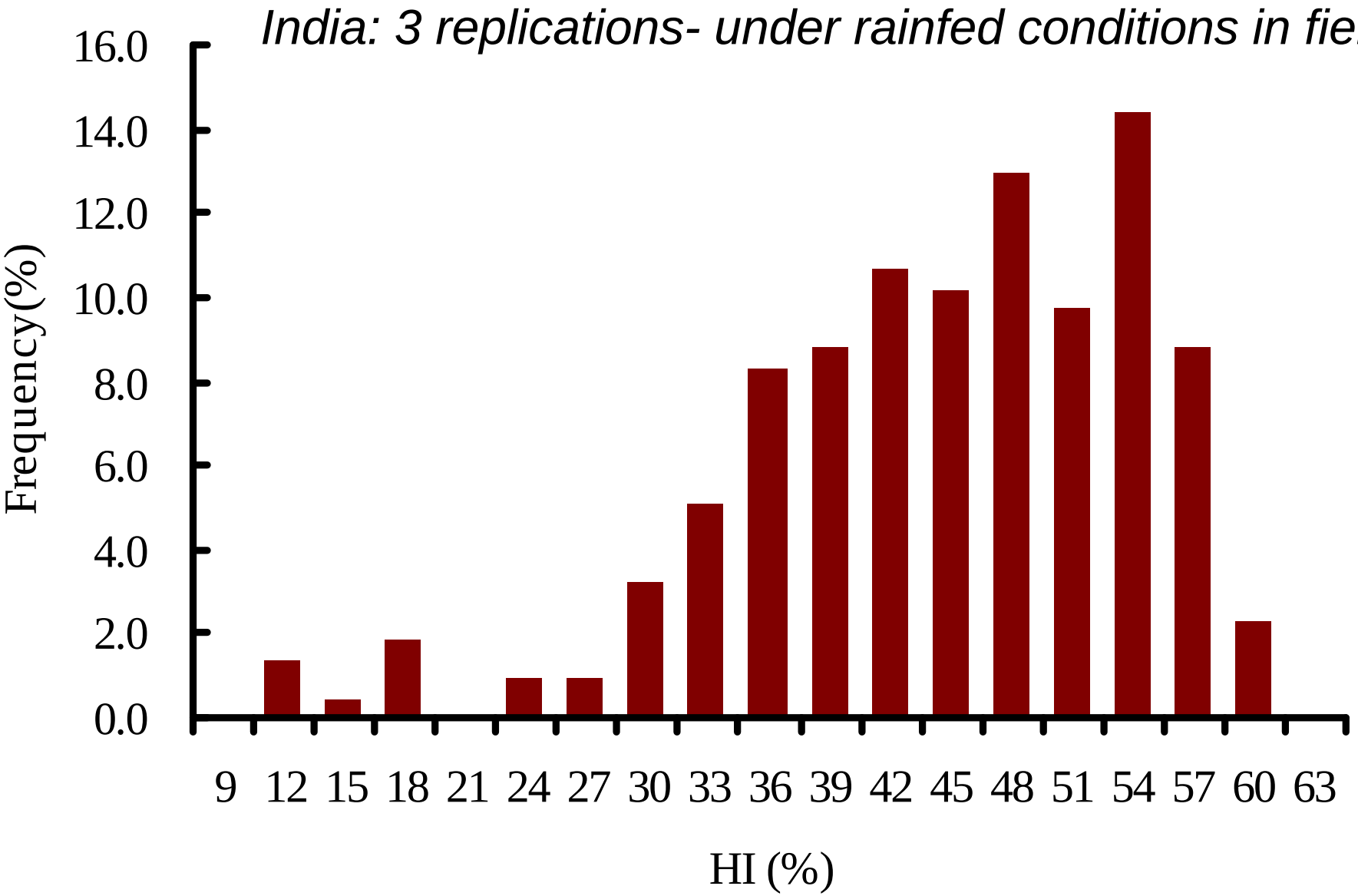
Evaluating the reference set…

Root traits

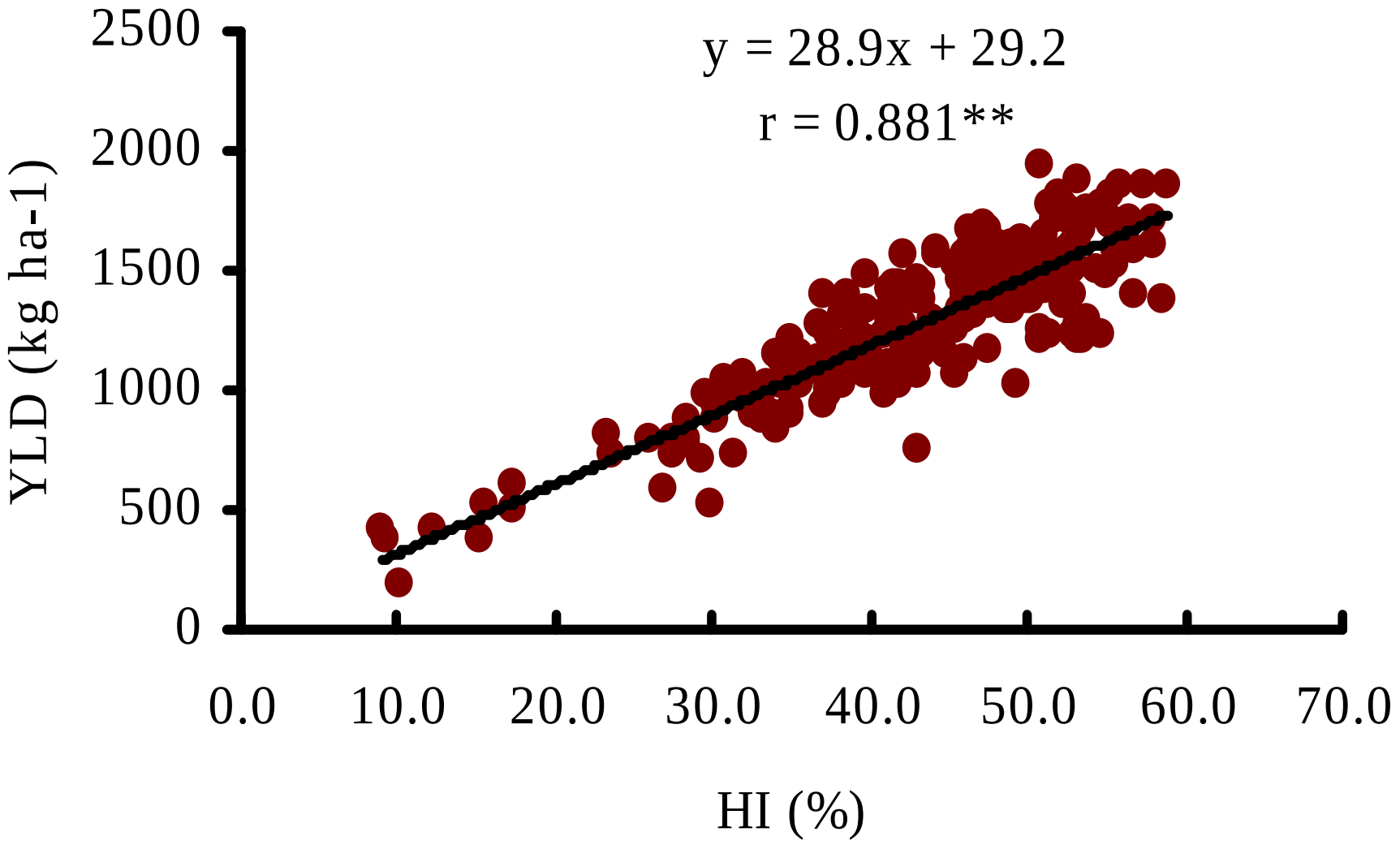
India
2 replications,
rainfed conditions,
total length of
all roots of the plant



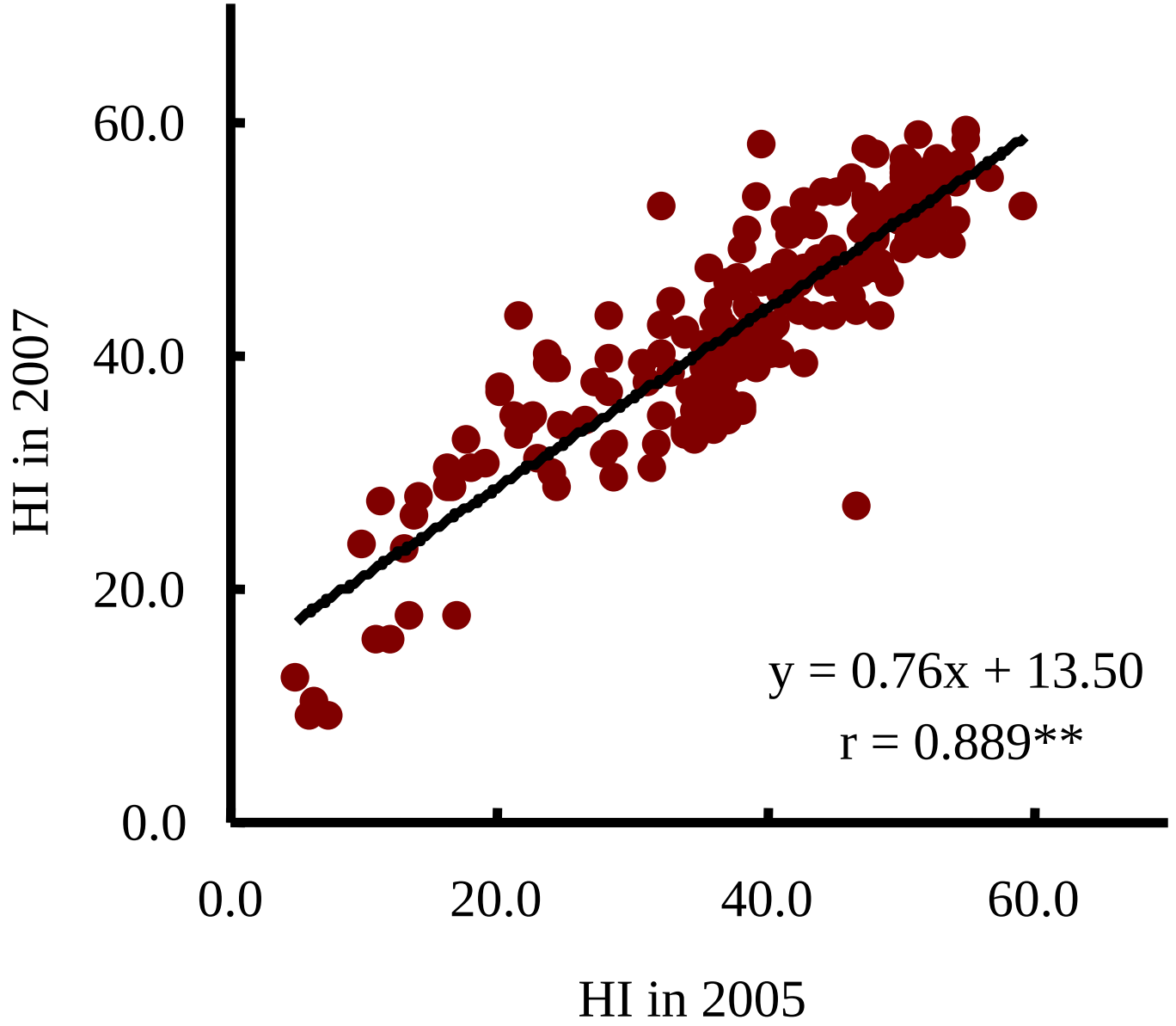
Harvest index (HI)



HI and yield


y = 28.9x + 29.2
r = 0.881**
YLD (kg ha-1)
2500
2000
1500
1000
500
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
HI (%)

HI data for two seasons



Large HI

ICC 4958

ICC 13863

ICC 6816

ICC 6279

ICC 13124

ICC 10393

Chafa

ICC 1398

Annigeri

ICC 4918

Helicoverpa resistance

Under natural infestation in field conditions

Genotypes	Damage rating	*Helicoverpa* larvae/ 10 plants		Overall resistance score	Pod damage (%)	Yield (q/ha)
		Vegetative	Flowering			
Ref Collection	4.4 (1.5 - 7.0)	14.1 (3.5 - 38.0)	4.5 (0.0 - 13.5)	5.8 (2.5 - 8.0)	18.1 (2.5 - 66.4)	7.5 (0.1 - 18.5)
ICC 506 (R)	1.0	9.5	0.0	4.0	7.3	7.6
ICC 3137 (S)	6.0	15.5	7.0	7.0	20.8	7.4
SE±	0.83	6.46	3.15	0.80	8.55	3.20
LSD (P = 0.05)	1.63	12.71	6.19	1.58	16.83	6.31

Helcoverpa nursery constituted:

: 10 best lines from reference collection

: 5 lines from interspecific mapping populaqtion

: 5 adavnaced *Helicoverpa* resistant breeding lines

: 5 high yielding lines with tolerance to *Helicoverpa*

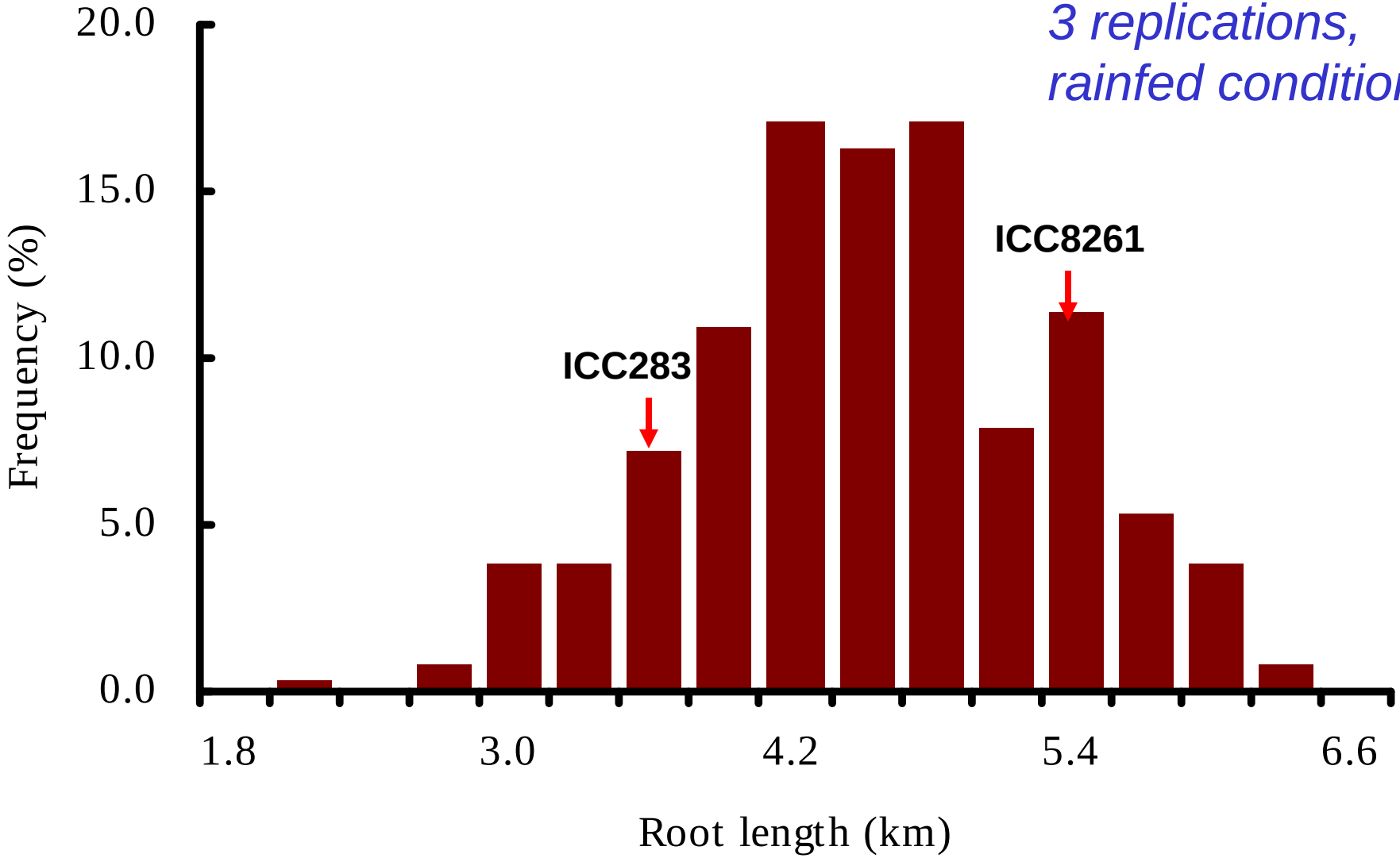
: 2 resistant, 2 susceptible and 1 local check

ICRISAT
Science with a human face

Mapping of root traits…

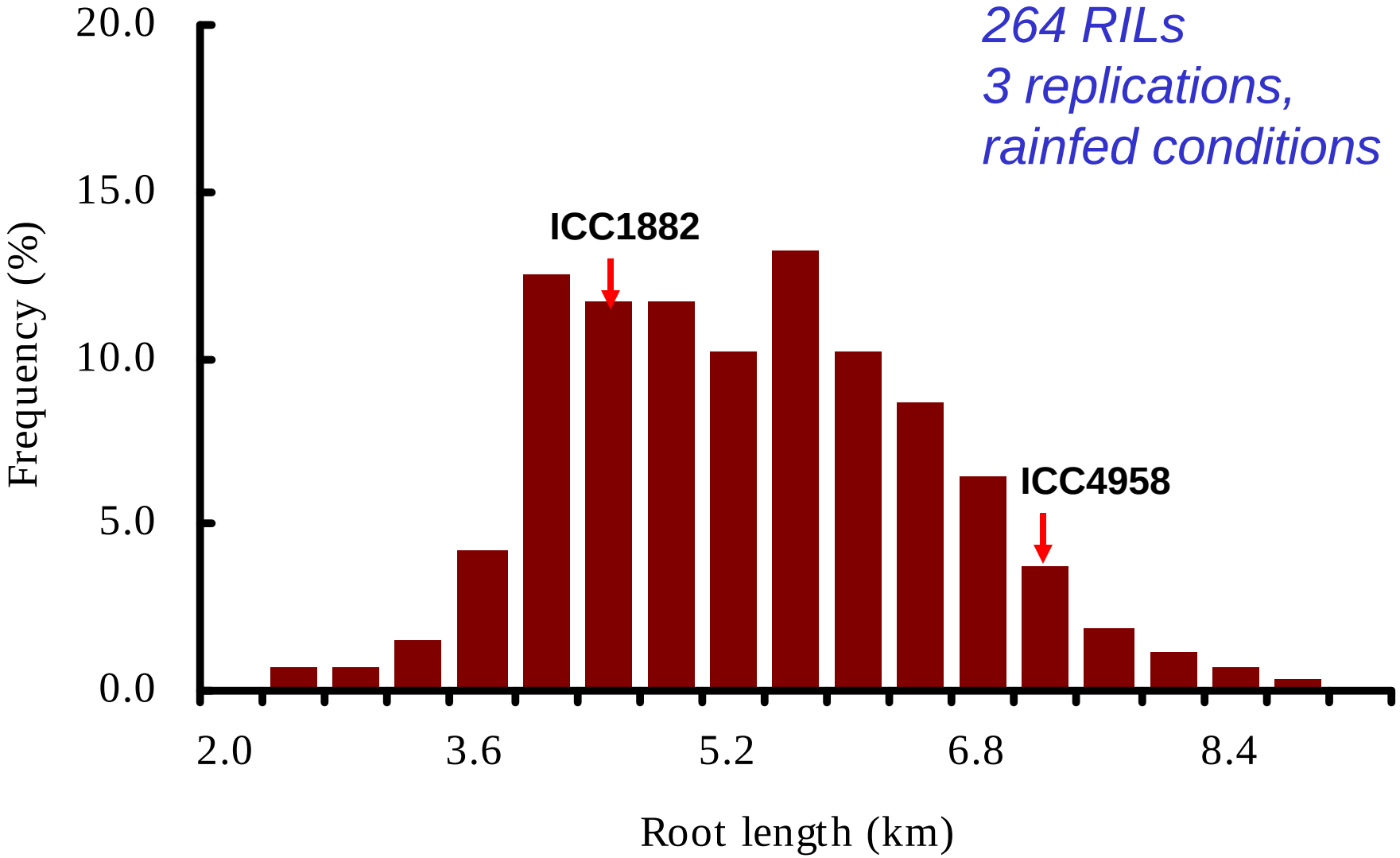
Phenotyping of ICC8261 x ICC283 for root traits (Year 2007)

281 RILs,
3 replications,
rainfed conditions



Phenotyping of ICC4958 x ICC1882 for root traits (Year 2007)

264 RILs
3 replications,
rainfed conditions



SSR marker polymorphism in ICC 4958 × ICC 1882

Markers	Total	Scorable	Polymorphic
H series	233	153	33
NIPGR	280	203	56
ICCM	311	225	23
CaM	1344	1214	~110
Winter series	241	183	~80

Available polymorphic SSR markers- 302

SSR marker polymorphism in ICC 283 × ICC 8261

Markers	Total	Scorable	Polymorphic
H series	233	153	42
NIPGR	280	203	82
ICCM	311	~170	~15
CaM	1344	1214	in progress
Winter series	241	-	in progress

Available polymorphic SSR markers- 139

ICRISAT
Science with a human face

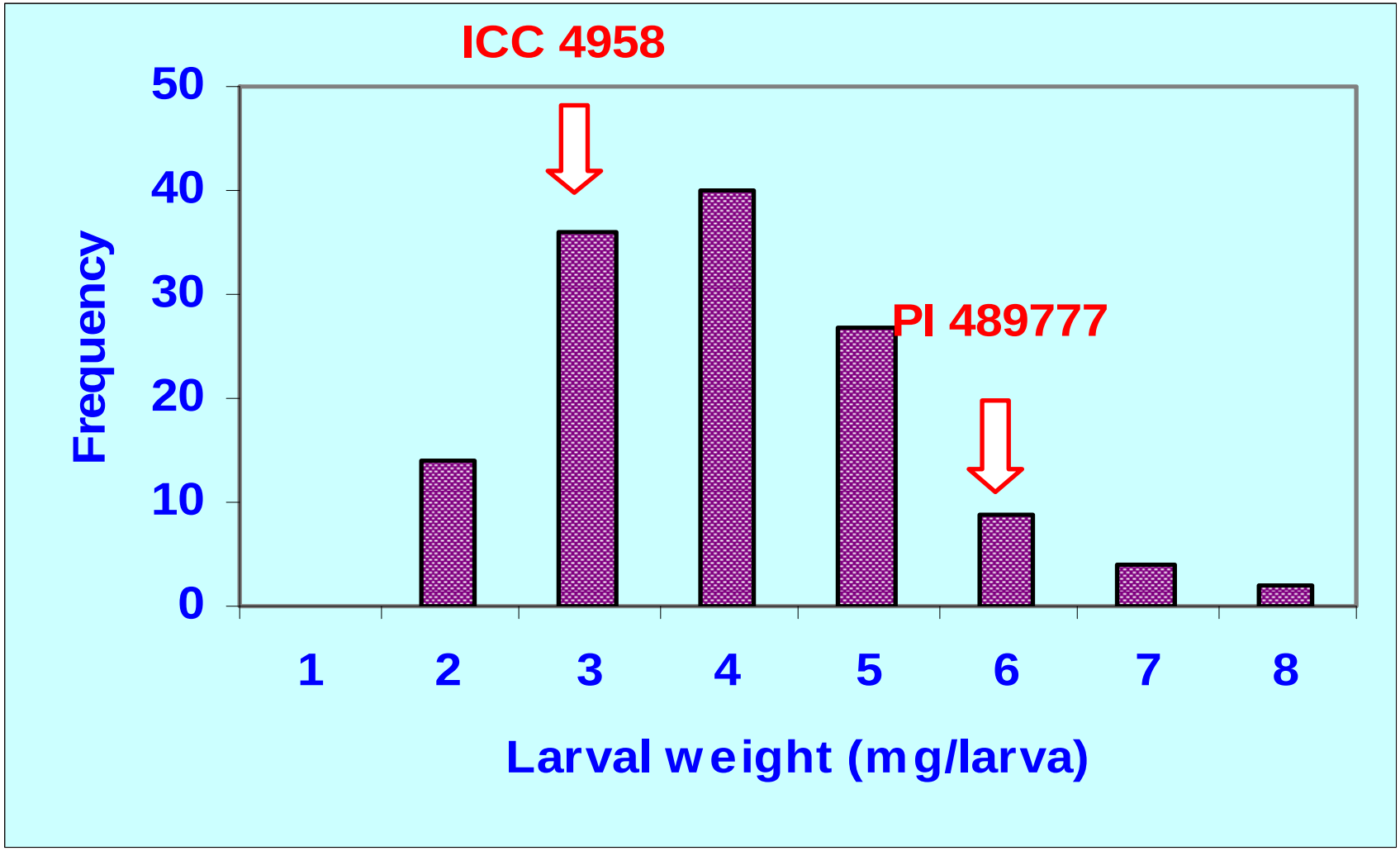
Mapping of insect resistance…

Insect resistance phenotyping of mapping population in field

131 RILs from C. arietinum ICC 4958 x *C. reticulatum* PI489777

Mapping population	Damage score at vegetative	*Helicoverpa* eggs/ 10 plants		*Helicoverpa* larvae/ 10 plants	
		Vegetative	Flowering	Vegetative	Flowering
Population	3.4 (1.5 - 6.5)	0.7 (0.0 - 8.5)	0.1 (0.0 - 2.0)	2.8 (0.0 - 8.0)	16.6 (4.0 - 40.0)
ICC 4958 (P1)	2.0	0.0	0.0	2.5	21.5
PI 489777 (P2)	5.5	0.5	0.0	1.0	12.5
ICC 506 (R)	1.0	0.0	0.0	1.5	5.5
ICCC 3137 (S)	2.5	1.0	0.0	7.5	39.5
ICCC 37 (S)	2.5	0.0	0.0	3.0	14.0
LSD (P = 0.05)	1.83	2.43	0.66	4.49	14.77

Screening of mapping population using detached leaf assay



Genotyping the insect resistance mapping population

(ICC 4958 × PI 489777)

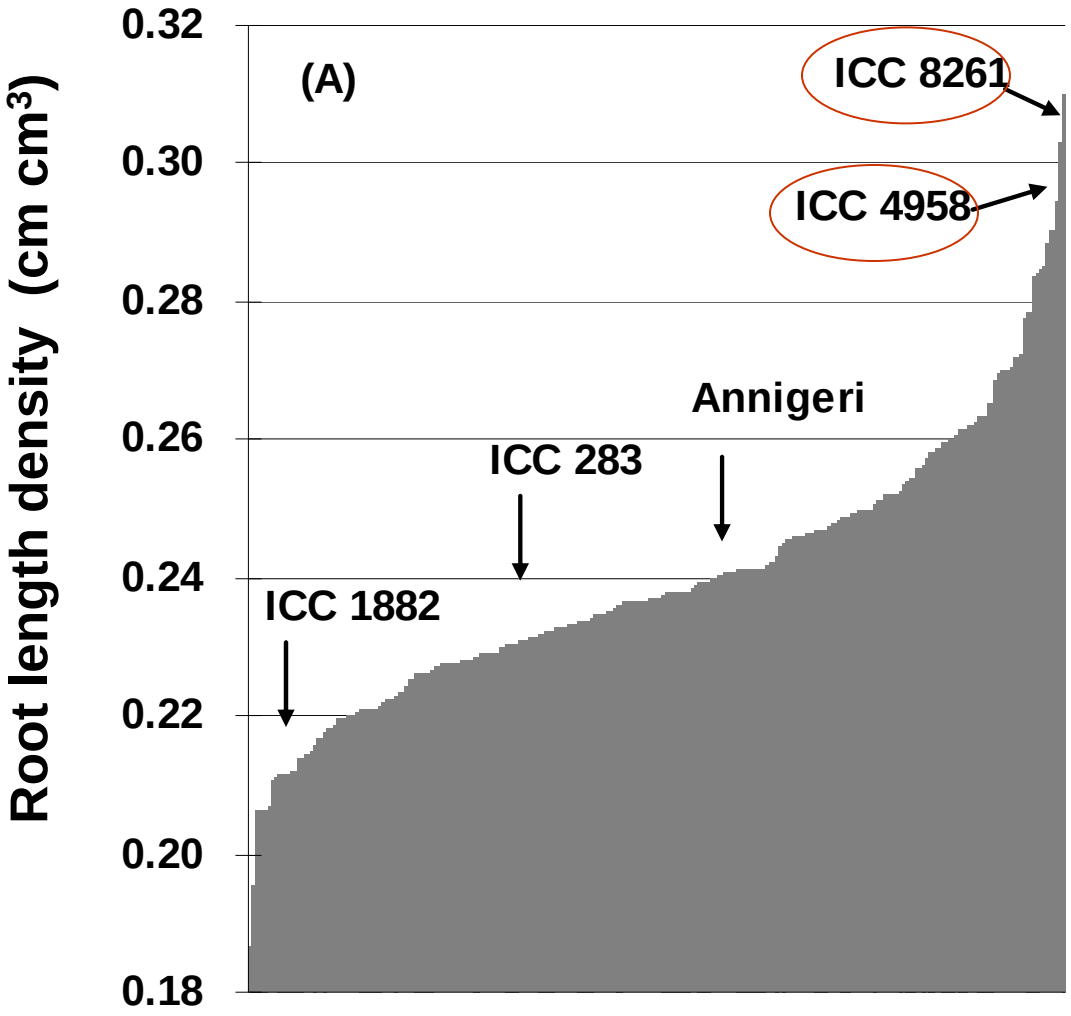
Markers	Total	Scorable	Polymorphic
H series	233	153	58
NIPGR	280	203	128
ICCM	311	225	52
CaM	1344	1214	252

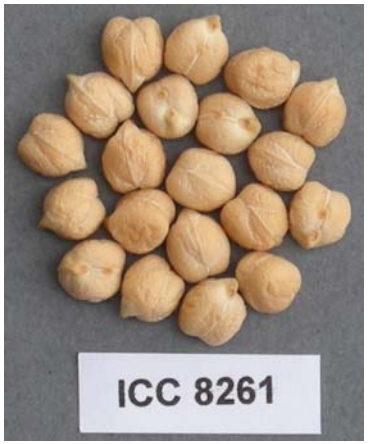
Total polymorphic- 490

Gene-based markers (>500) are being mapped under Objective 5

Modern breeding: MABC & MARS...

Donor parents for MABC

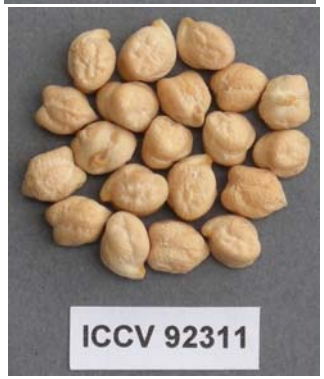




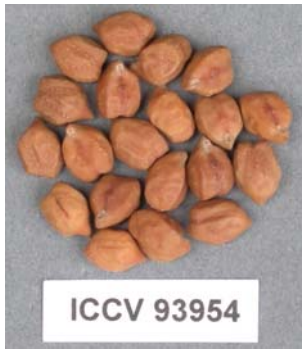
Recurrent parents for introgression of root traits

Three cultivars were selected

ICCV 92318 (kabuli type): Released as Chefe in Ethiopia and as Hawata in Sudan and performing well in Kenya and Tanzania



ICCV 92311 (kabuli type): Released as KAK 2 in India and performing well in Kenya and Tanzania.



ICCV 93954 (desi type): Released as JG 11 in India and expected to perform well in SSA

MABC initiated

Crosses made

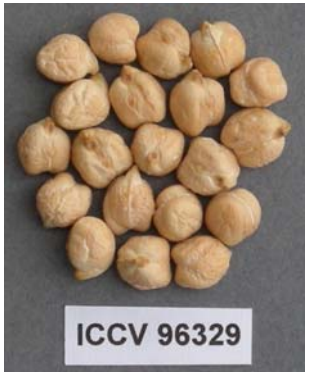
ICCV 92318 (kabuli) x ICC 8261(kabuli)
ICCV 92311 (kabuli) x ICC 8261(kabuli)
ICCV 93954 (desi) x ICC 4958 (desi)

BC1 made

ICCV 92318 x (ICCV 92318 x ICC 8261)
ICCV 92311 x (ICCV 92311 x ICC 8261)
ICCV 93954 x (ICCV 93954 x ICC 4958)

BC1F1 seeds available: > 100 seeds in each cross

Cultivars for MARS



ICCV 2 (kabuli): Released as Swetha in India, Wad Hamid in Sudan and Yezin 3 in Myanamr

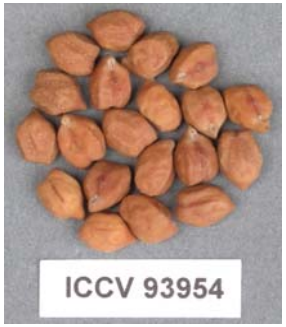
ICCV 93954 (desi): Released as JG 11 in India and expected to perform well in SSA

ICCV 92318 (kabuli): Released as Chefe in Ethiopia and Hawata in Sudan and performing well in Kenya and Tanzania

ICCV 96329 (kabuli): Released as LBeG7 in India and performing well in Kenya

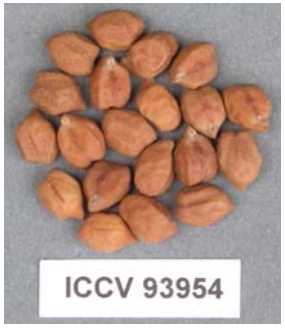
ICCV 97105 (desi): Performing well in Kenya

Crosses made for MARS



ICCV 2 x ICCV 93954
500 F2 generated
200 F2 being genotyped

ICCV 92318 x ICCV 96329
F1 grown



ICCV 97105 x ICCV 93954
F1 grown

Significant Achievements (in Year 1)

- More than 1500 SSRs developed- enhanced the repertoire of chickpea SSRs to >2000
- Reference collection phenotyped for root traits, HI and insect resistance
- Two mapping populations phenotyped for root traits and 200-400 polymorphic SSR markers identified
- Interspecific mapping population phenotyped for insect resistance and about 500 new polymorphic SSR markers identified
- MABC and MARS activities initiated

Chickpea Team

Dave Hoisington (PI- May 2007- May 2008)
Rajeev Varshney (PI- since June 2008/Leader-Activity 2)
Said Silim/ N GangaRao (Leader- Activity 1)
Hari Sharma (Leader- Activity 3)
Junichi Kashiwagi (Leader- Activity 4)
Pooran Gaur (Leader- Activity 5)

NARS:

Paul Kimurto, Kenya
Million Eshete, Ethiopia
Robert Kielo, Tanzania
Massod Ali, IIPR, India
Jayashree, UAS, India
Jayalakshmi, ANGRAU, India

Collaborators:

Hari Upadhyaya, ICRISAT
Doug Cook, USA
Sabhyata Bhatia, India
Peter Winter, Germany


Thanks for your kind attention
Science with a Human Face